1 # Transcriptional signatures of invasiveness in *Meloidogyne*

2 # *incognita* populations from sub-Saharan Africa

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11 ## Abstract

12 *Meloidogyne incognita* is an economically important plant parasitic nematode. Here we

13 demonstrate substantial variation in the invasiveness of four *M. incognita* populations relative to

14 tomato. Infective (J2) stage transcriptomes reveal significant variation in the expression of protein-

15 coding and non-coding RNAs between populations. We identify 33 gene expression markers (GEMs)

16 that correlate with invasiveness, and which map to genes with predicted roles in host-finding and

17 invasion, including neuropeptides, ion channels, GPCRs, cell wall-degrading enzymes and microRNAs.

18 These data demonstrate a surprising diversity in microRNA complements between populations, and

19 identify GEMs for invasiveness of *M. incognita* for the first time.

20

21 Key words*:* Root-knot nematode*,* behaviour, invasion, transcriptome, microRNAs, plant parasitic

22 nematode.

23

24 *Meloidogyne incognita* is a globally distributed and highly polyphagous parasite of crop plants

25 (Coyne et al., 2018; Trudgill & Blok, 2001), demonstrating a surprisingly high level of adaptive

26 variability for an asexual organism (Szitenberg et al., 2017). This adaptability is thought to play a role

27 in the pests' ability to rapidly evade sources of crop resistance. Consequently, *M. incognita* is

28 becoming increasingly problematic, and current approaches to control are insufficiently robust or

29 durable to provide reliable protection in the field (Davies & Elling, 2015). The natural variation

30 between *M. incognita* populations is poorly understood (Bucki et al., 2017). This constitutes a

31 substantial gap in our knowledge, which could hinder our ability to develop sources of durable

32 resistance to field populations. The relatively high work burden of population maintenance in the

33 laboratory, and inevitable domestication of *M. incognita* populations makes the assessment of field-

34   relevant inter-species variation a significant and ongoing technical challenge. In addition, access to

35   populations that are native to Nagoya protocol (https://www.cbd.int/abs/) signatories can be

36   problematic. Whilst the Nagoya protocol aims to promote equitable commercial outcomes arising

37   from native genetic resources, opportunities for collaboration and extended sharing of resources are

38   limited. Collectively, these challenges promote an artificial over-reliance on highly domesticated

39   legacy strains, which are unlikely to reflect the genotypic or phenotypic spectra of field populations.

40   Although there are many potential approaches to developing crop parasite resistance, an

41   improved understanding of parasite host-finding and invasion may facilitate the development of

42   new strategies that prevent infection. This is preferable to sources of resistance that are active *in*

43   *planta*, as it limits the opportunity for secondary pathogen infection, and minimises the metabolic

44   burden of mounting a defence response to invading parasites. In this study, we assessed the host-

45   finding and invasion behaviour of *M. incognita* populations that had been recently collected from

46   field sites in Kenya and Nigeria, with the aim to relate observed behavioural variation to gene

47   expression signatures using transcriptomic correlation. These data would improve our

48   understanding of the link between genotype and phenotype, which may enable us to identify new

49   targets for nematicide development, or biotechnological intervention.

50   We considered three populations collected from Nigeria, named Nig_1, Nig_2, and Nig_3,

51   and one population from Kenya, named Ken_1. Our data demonstrate statistically significant

52   variation in the propensity of these *M. incognita* populations to invade tomato cv. Moneymaker

53   seedlings. Nig_1 is the most invasive, with a mean of 50.06 ±7.9 J2s (from a total of 200 J2s) invading

54   within 24 h, followed by Ken_1 with a mean of 39.9 ±6.9, Nig_2 with a mean 27.3 ±7.6, and Nig_3

55   being the least invasive, with a mean of 2.5 ±0.8 J2s invading within 24 h (Figure 1).

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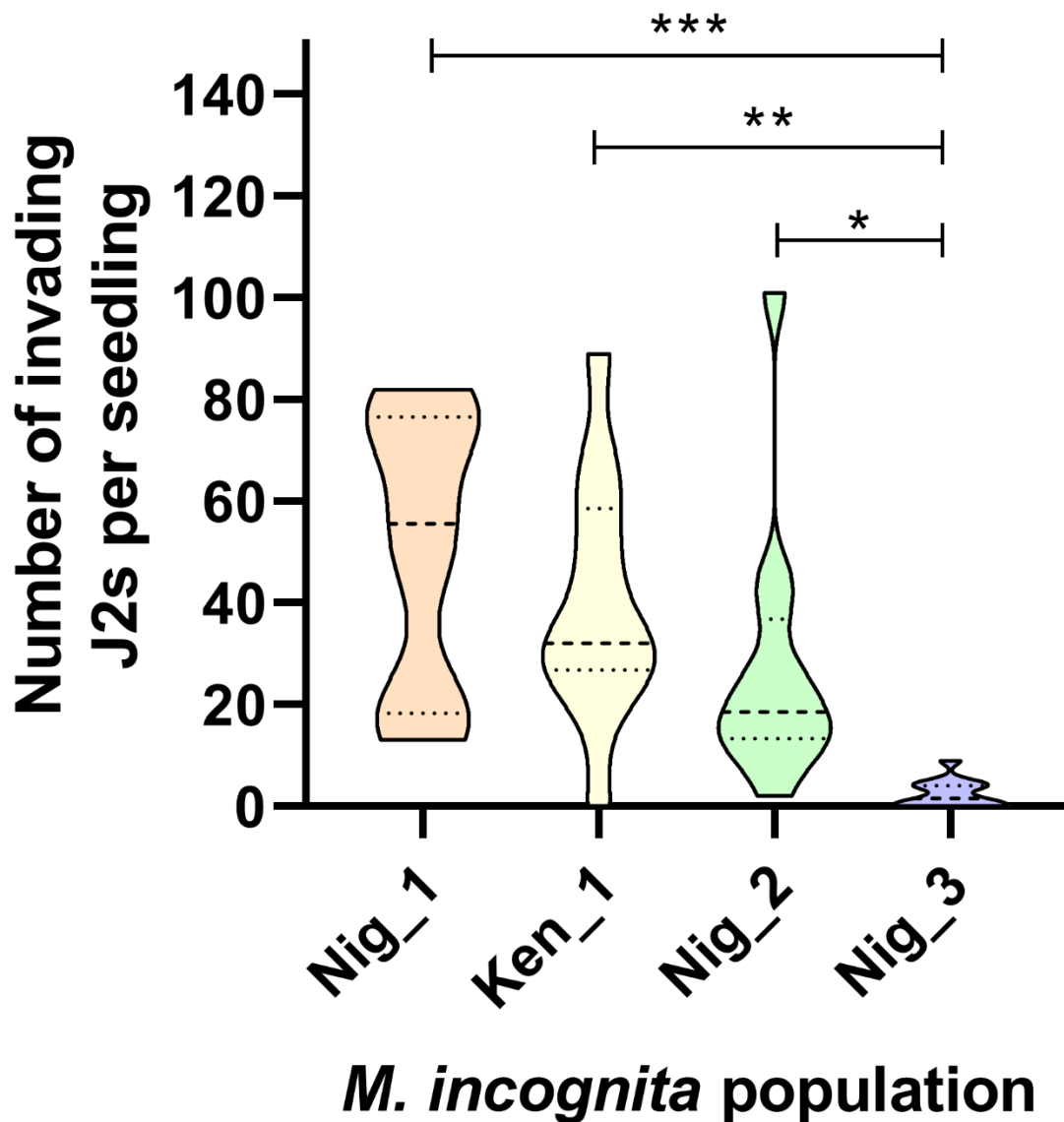
Figure 1. *Meloidogyne incognita* invasion of tomato cv. Moneymaker seedlings is highly variable.

Violin plot showing number of J2s invading tomato seedlings, 24 h post exposure. Dashed lines

indicate the median, dotted lines indicate the quartiles. Data assessed by ANOVA and Tukey's

multiple comparison test using Graphpad Prism 8; $P<0.05*$, $P<0.01**$, $<0.001***$. *M. incognita*

populations were collected from field sites in Kenya and Nigeria. They were cultured on tomato cv.

Moneymaker, in plant growth cabinets at 23°C, with a regular 16 h light, 8 h dark cycle for no more

than two generations following field collection. Tomato seedling infection assays were conducted as

in Warnock et al. (2016), using 200 J2s per seedling, inoculated into an agar slurry containing the

tomato seedling.

66

We conducted high-throughput sequencing of protein-coding and non-coding RNAs from the

68   infective J2 stage of each *M. incognita* population to understand the molecular basis of behavioural

69   variation. Our data revealed that up to 6,232 (13.7%) transcripts were significantly up-regulated

70   (P<0.0001****) relative to the most invasive population, Nig_1, with up to 4,908 (10.8%) down-

71   regulated across pairwise comparisons (Figure 2A; supplemental file S1). Using mirDeep2, we

72   identified 192 precursor microRNA genes across the four *M. incognita* populations, relating to 144

73   predicted mature microRNAs in *M. incognita* Nig_1; 146 in Nig_2; 105 in Nig_3; and 176 in the Ken_1

74   population. This constitutes a surprising diversity in microRNA complement between populations of

75   the same species, with Ken_1 representing the major outlier, with 44 predicted mature microRNAs

76   unique to that population (supplemental file S2). By way of comparison, a similar analysis using the

77   entomopathogenic nematode *Steinernema carpocapsae* revealed variation from 269 to 273

78   predicted mature microRNAs across three populations (Warnock et al., 2018). Up to 52 (27%) of the

79   predicted and conserved *M. incognita* microRNA genes were significantly up-regulated

80   (P<0.0001****) relative to the most invasive Nig_1 population, with up to 23 (12%) down-regulated

81   across pairwise comparisons (Figure 2B).

82   We populated a list of Gene Expression Markers (GEMs) that correlated, either positively or

83   negatively, with the observed invasion phenotypes. This was achieved by arranging the population

84   comparisons from most invasive to least invasive (Nig_1 vs Ken_1; Nig_1 vs Nig_2; Nig_1 vs Nig_3),

85   and constraining gene lists to those that followed expression patterns consistent with the

86   phenotypic trend. Correlating GEMs were identified when the log2 fold change quotients between

87   adjacent comparisons were greater than one, with at least a P<0.05* difference between each

88   population, and at least P<0.0001*** between the most and least invasive populations. Using this

89   approach, we identified 485 GEMs that correlate with the observed invasion phenotype of *M.*

90   *incognita*, comprising 483 protein-coding genes, and two microRNA genes; 242 GEMs correlate

91   positively with the invasion phenotype, and 243 GEMs correlate negatively (Supplemental Files S1 &

92   S2). On inspection of the invasion GEM list, we identified a total of 33 genes with predicted roles in

93   the regulation of host-finding and invasion behaviour, including genes associated with the

94   neuropeptidergic system, neuronal signalling, cell wall-degrading enzymes, and the two microRNA

95   genes (Figure 2C). It is possible that other correlating genes play a functional role in the invasion

96   phenotype, however we deemed that these 33 genes were most likely to exert the largest influence,

97   based on known or predicted functionality.

98   Six neuropeptide genes correlated positively with *M. incognita* invasiveness, including

99   *FMRFamide-like peptide 14ii (flp-14ii), flp-16ii, flp-19ii, flp-32i INSulin-like protein 3ii (ins-3ii)*, and

100   *Neuropeptide-Like Protein 46i (nlp-46i)*. Three neuropeptide genes, *flp-14i*, *nlp-13i* and *nlp-55i* were

101   negatively correlated with the invasion phenotype (Figure 2C). Expression of a predicted

102   prohormone convertase chaperone, *sbt-1*, which is required for the biosynthesis of neuropeptides in

103   the free-living nematode *Caenorhabditis elegans* (Husson & Schoofs, 2007)*,* also correlated positively

104   with invasion behaviour. These data implicate the neuropeptidergic system, and FLPs in particular, in

105   the modulation of *M. incognita* invasion behaviour. This corroborates previous observations of *flp*

106   gene enrichment within the infective juvenile stage of many parasitic nematode species, and a role

107   in the behavioural diversification of these stages (Lee et al., 2017). Indeed, our own work

108   demonstrates similar associations between neuropeptidergic genes and the host-finding behaviour

109   of *S. carpocapsae* (Warnock et al., 2018; Morris et al., 2017). Four putative neuropeptide G-Protein

110   Coupled Receptor (GPCR) genes were also found to associate with *M. incognita* invasiveness, along

111   with seven ion channel genes, two innexin genes, an acetylcholinesterase and a predicted *odr-8*

112   peptidase homologue (Figure 2C). Within the 485 correlating GEMs, we also identified 62 novel

113   genes, with no known function, or orthology to *C. elegans* genes (supplemental file S1). Six plant Cell

114   Wall-Degrading Enzyme (CWDE) genes were also associated positively with invasion phenotypes,

115   corresponding to four *endo-β-1,4-glucanase* genes, and two predicted pectate lyase (*pel*) genes

116   (Figure 2C). Each CWDE gene is most highly expressed in the most invasive Nig_1 population, and

117   display lowest expression in the least invasive population, consistent with a role in mediating the

118   enzymatic degradation of the plant cell wall. If it can be demonstrated that certain CWDEs confer a

119   specific advantage for the invasion of particular host species, it could point to new approaches to

120   resistance based on the modification of cell wall composition, potentially in conjunction with recent

121   developments in synthetic biology.
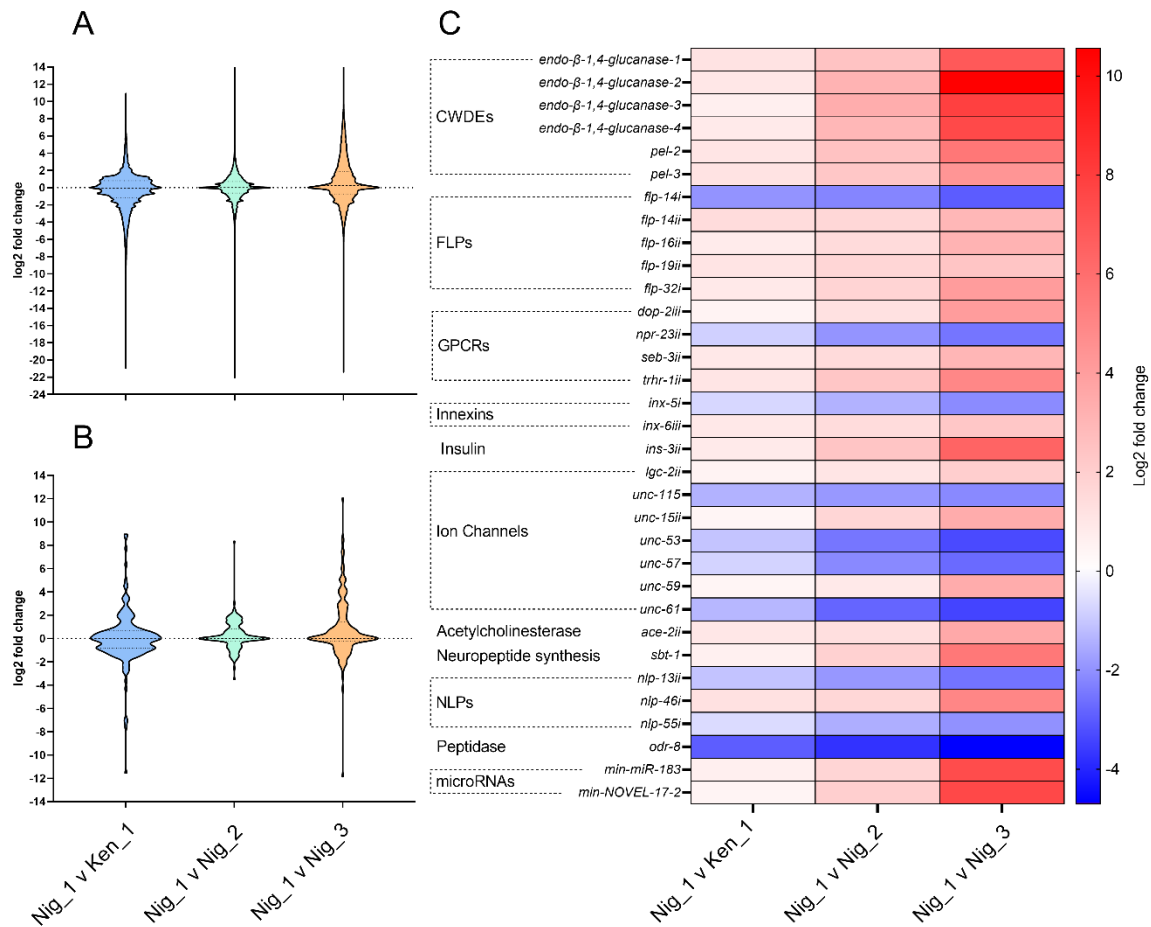
M. incognita population comparisons

Figure 2. Transcriptomic signatures of *Meloidogyne incognita* invasiveness. Violin plots of log2 fold changes across pairwise population comparisons for (A) protein-coding genes and (B) microRNAs. (C) Summary heatmap of 33 selected GEMs, demonstrating the log2 fold change between pairwise comparisons, relative to the most invasive Nig_1 population. GEMs follow a gradient expression pattern that positively or negatively correlates with the population invasion phenotype; CWDEs – Cell Wall Degrading Enzymes. Figures were generated in Graphpad Prism 8. RNA extraction, library preparation, sequencing, bioinformatics and statistical analyses were conducted as in Warnock et al. (2018). Briefly, ~3000 J2s of each *M. incognita* population were used to extract total RNA, from which coupled 150 bp paired-end, and 50 bp single end illumina HiSeq libraries were prepared for each population, in triplicate. Libraries were sequenced on one illumina HiSeq 2500 lane. Following quality control, reads were mapped to the most recent *M. incognita* genome assembly (PRJEB8714, WBPS12, https://parasite.wormbase.org) using STAR and RSEM (Blanc-Mathieu et al., 2017; Howe et al., 2015; Dobin et al., 2013; Li et al., 2011). MicroRNAs were identified and quantified using MirDeep2 (Friedländer et al., 2012). Predicted microRNAs were named using a BLAST search against *C. elegans* microRNAs (www.miRBase.org), and previously identified *M. incognita* microRNAs (Zhang

138   et al., 2015). Predicted microRNAs were named in line with *C. elegans* or *M. incognita* microRNAs

139   represented as the top BLAST return, and if there was a sequence identity match greater than 80%.

140   All novel *M. incognita* microRNAs were named sequentially, ensuring no overlap with names

141   allocated to *C. elegans* or previously published *M. incognita* microRNAs. Predicted microRNA target

142   genes were identified with MiRanda (Enright et al., 2003), using strict and unrestricted discovery

143   modes, as in Warnock et al. (2018). Differentially expressed protein-coding and non-coding genes

144   were identified using DESeq2 (Love et al., 2014). All sequencing datasets are available from the SRA

145   database (Bioproject: PRJNA525879).

146

147   Non-coding microRNAs have been implicated in nematode behavioural variation (Warnock et al.,

148   2018; Rauthan et al., 2017; reviewed in Ambros & Ruvkun, 2018), through the regulation of target

149   gene expression. Our data reveal that the expression of two mature microRNAs correlates with the

150   invasion phenotype of *M. incognita* populations (Figure 2C). Using miRanda to identify predicted

151   gene targets, in both strict and unrestricted settings, reveals a surprising abundance, and inter-

152   connection between these microRNAs and neuropeptide genes, spanning the *flp*, *nlp* and *ins*

153   families, in addition to GPCR and ion channel genes (supplemental file S3, S4). It has been suggested

154   that microRNAs regulate developmental programmes through the coordinated and cooperative

155   targeting of genes involved in specific biological functions (Zhang et al., 2009). Our data indicate that

156   this may also be the case for behavioural regulation. For example, *Min-NOVEL-17-2* is predicted to

157   simultaneously target: *flp-1i, flp-1ii, flp-11i, flp-11ii, flp-11iii, flp-33i, flp-33ii, flp-34i, ins-1i, ins-1ii, ins-*

158   *1iv, ins-1v, ins-18i, nlp-8ii, nlp-12, nlp-13i, nlp-13ii, nlp-81iii*, in addition to a variety of other ion

159   channel, GPCR and innexin genes (supplemental file S5). To further investigate the potential

160   relationship between microRNAs and behavioural regulation, our analysis sought to identify

161   predicted interactions that followed the expected trend for biologically interacting microRNA-

162   mRNAs, at the level of mRNA abundance. A substantial literature has developed around microRNA

163   induced mRNA decay in animals (reviewed in Iwakawa and Tomari, 2015), and our data demonstrate

164   a correlation between numerous predicted microRNA targets, and expression patterns between

165   populations, indicating that these microRNA-mRNA interactions may be biologically relevant. For

166   example, *nlp-13ii* is identified as both an *in silico* predicted target of the novel microRNA *Min-*

167   *NOVEL-17-2* and is demonstrated to follow an expression pattern consistent with microRNA-

168   mediated mRNA decay across populations (Figure 3A). However, whilst *nlp-13i* is also a predicted

169   target of *Min-Novel-17-2*, it does not follow an expression trend that is consistent with microRNA-

170   mediated decay. This indicates either that the *nlp-13i* and *nlp-13ii* transcripts are expressed in

171   different cells / tissues that only partially overlap with expression of *Min-NOVEL-17-2,* or that there

172   are other transcript-specific features, which influence the tendency towards microRNA-mediated

173   mRNA decay or translational inhibition. One possible explanation relates to altered secondary

174   structure of UTR sequences, which may underpin differences in the bioavailability, or function of

175   microRNA target sites. Based on *in silico* structural predictions using the Vienna RNAfold server

176   (http://rna.tbi.univie.ac.at/), this does not appear to be a factor for the UTRs of *nlp-13* gene copies

177   at least. Our analysis in Figure 3 focuses solely on predicted microRNA interactions with the 483

178   protein-coding genes identified as invasion GEMs, and on that basis makes no judgement on the

179   likelihood of interactions with the many other predicted target genes listed above, which do not

180   follow the stringency criteria used to populate the list of GEMs.

M. incognita population comparisons

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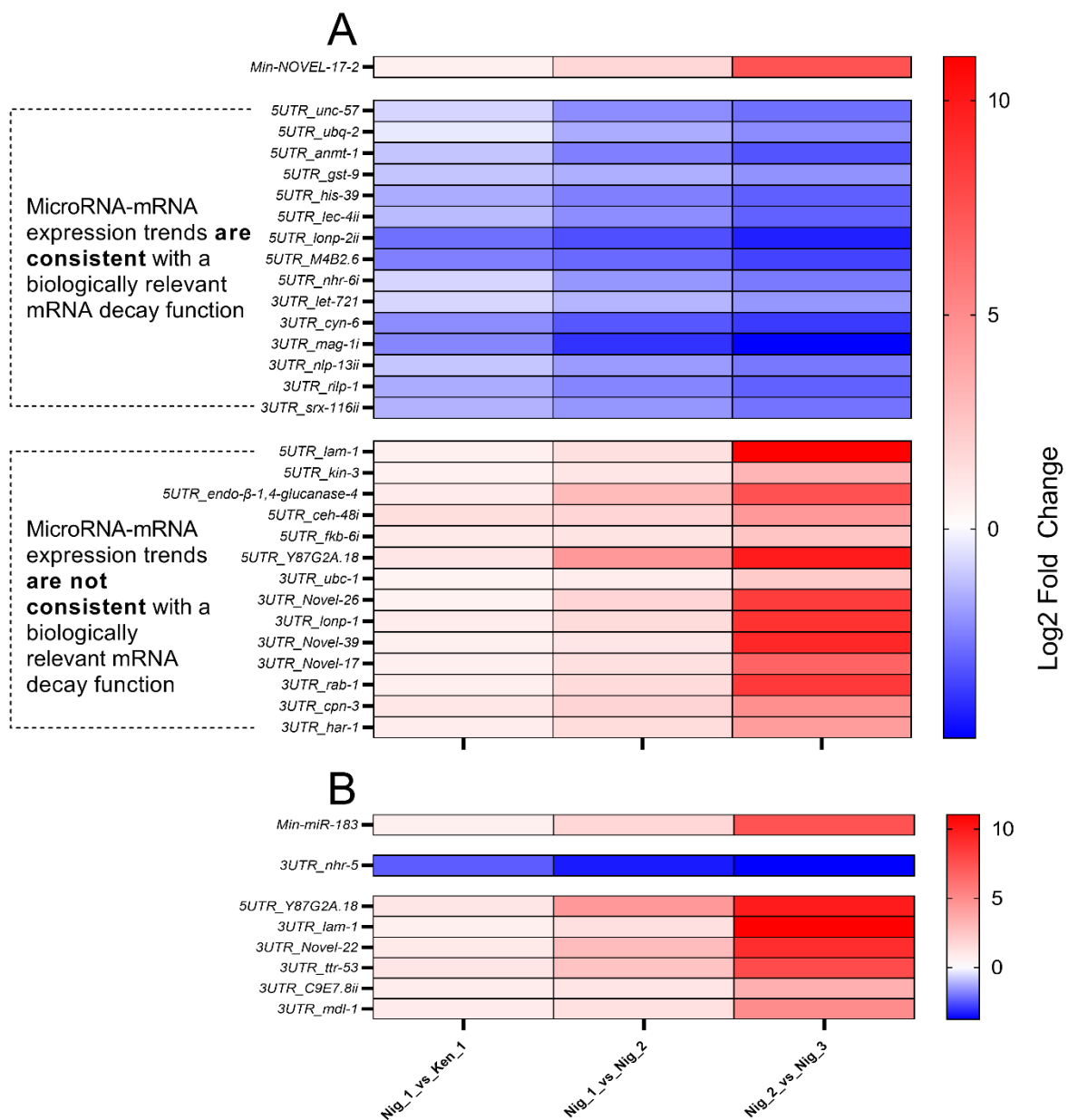
182 **Figure 3. Differential expression of predicted microRNA targets suggests biologically relevant**

183 **interactions across *Meloidogyne incognita* populations.** (A) Heatmap demonstrating differential

184 expression trends for the microRNA, *Min-NOVEL-17-2,* and predicted mRNA targets identified within

185 the list of 483 protein-coding GEMs. The location of the predicted microRNA interaction point is

186 indicated as a 5'UTR or 3'UTR suffix. 15 genes negatively correlate with *Min-NOVEL-17-2* changes

187 across pairwise population comparisons, which suggests biologically relevant interactions, mediated

188 through mRNA decay. 14 of the predicted targets, which follow the phenotype trend, do not

189 correlate with *Min-Novel-17-2* in a way that suggests a biologically relevant interaction. (B) Heatmap

190 demonstrating differential expression trends for the microRNA, *Min-miR-183* and predicted mRNA

191 targets identified within the list of 483 protein-coding GEMs. Only one of the predicted target genes

192 negatively correlates with microRNA differential expression trends, suggesting that the putative

193 nuclear hormone receptor gene, *nhr-5*, is the only biologically relevant target within this set of

194 invasion GEMs. The microRNA target analysis presented here is not intended to be an exhaustive

195 treatment of all predicted mRNA interactions, focusing instead on the GEMs identified through

196 transcriptomic and phenotypic correlation.

197

198 Apomictic *Meloidogyne* spp. are known to possess highly divergent hypotriploid genomes, with

199 multiple variant gene copies (Szitenberg et al., 2017). The sequence variation of gene copies may

200 reflect the functional diversification of common genetic elements for adaptive purposes, through

201 hybridisation and selection. For example, a significant number of putative neuropeptide gene copies

202 are found to encode the same predicted mature neuropeptide(s) within a variant mRNA sequence;

203 such genes are identified within this manuscript by virtue of a Roman numeral suffix, assigned

204 according to the order of discovery. Expression of *flp-14ii* correlates positively with increased

205 invasiveness, whereas *flp-14i* correlates negatively with increased invasion behaviour (Figure 3, and

206 Supplemental File S1). Whilst we have no insight to the relative role or function of gene copies, or if

207 these copies co-localise, we have established that they can be differentially expressed, within and

208 between populations of the same species. Our analysis of predicted microRNA interactions reveals a

209 considerable amount of variation in the predicted 5' and 3' UnTranslated Regions (UTRs) of

210 predicted neuropeptide gene copies, which underpins qualitative and quantitative variation in

211 predicted microRNA targeting (supplemental files S3, S4 and S5). UTR sequence variation has

212 received little attention in the literature for parasitic nematode species, however UTR sequences are

213 known to be highly variable across developmental stages, and tissues of the model *C. elegans*, which

214 drives the genic regulation of microRNA interactions (Blazie et al., 2010; Mangone et al., 2010). It is

215 possible that the hypervariation of gene copy UTRs between *M. incognita* populations could be

216   adaptive, driving functional divergence as a factor of differential microRNA targeting. Data support a

217   similar hypothesis for UTR isoform variation and behavioural diversification of *S. carpocapsae* strains

218   (Warnock et al., 2018). This could provide a functional explanation for the extraordinary variation

219   and adaptiveness of apomictic *Meloidogyne* spp.

220   This study demonstrates a surprising behavioural variation amongst *M. incognita*

221   populations that are native to Kenya and Nigeria and provides the first evidence of GEMs that

222   correlate with the invasion phenotype. Furthermore, we observe substantial variation in the

223   complement of microRNA genes between populations, and variation in gene UTR targets between

224   variant gene copies, which could underpin behavioural adaptation to host and environment. These

225   observations require detailed functional studies to ascertain the specific influence of implicated

226   genes and microRNAs. Whilst the inevitable domestication of *M. incognita* populations under

227   laboratory and greenhouse conditions constitutes a technical challenge for the study of field-

228   relevant diversity and phenotype, we expect these populations to become better adapted to the

229   experimental host. This could provide opportunity to track signatures of molecular adaptation over

230   time, within an experimental evolutionary approach (reviewed by Kawecki et al., 2012).

231

232   **Acknowledgements**

237

238   **Supporting information captions**

239

240   Supplemental file S1. Global DESeq2 output across all pairwise population comparisons, and

241   complete list of invasion GEMs.

242   Supplemental file S2. List of predicted microRNAs and global DESeq2 output across all pairwise

243   population comparisons.

244   Supplemental file S3. MicroRNA target prediction analysis for global 5'UTRs.

245   Supplemental file S4. MicroRNA target prediction analysis for global 3'UTRs.

246

247   **References**

248   Ambros V, Ruvkun G (2018). Recent molecular genetic explorations of *Caenorhabditis elegans*

249   microRNAs. Genetics 209(3): 651-673. https://doi.org/10.1534/genetics.118.300291

250

251   Blanc-Mathieu R, Perfus-Barbeoch L, Aury JM, Da Rocha M, Gouzy J, Sallet E, Martin-Jimenez C,

252   Bailly-Bechet M, Castagnone-Sereno P, Flot JF, Kozlowski DK, Cazareth J, Couloux A, Da Silva C, Guy J,

253   Kim-Jo YJ, Rancurel C, Schiex T, Abad P, Wincker P3, Danchin EGJ (2017). Hybridization and

254   polyploidy enable genomic plasticity without sex in the most devastating plant-parasitic nematodes.

255   PLoS Genet. 13(6): e1006777. https://doi.org/10.1371/journal.pgen.1006777

256

257   Blazie SM, Geissel HC, Wilky H, Joshi R Newbern J, Mangone M (2017). Alternative polyadenylation

258   directs tissue-specific miRNA targeting in *Caenorhabditis elegans* somatic tissues. Genetics 206(2):

259   757-774. https://dx.doi.org/10.1534/genetics.116.196774

260

261   Bucki P, Paran I, Ozalvo R, Iberkleid I, Ganot L, Miyara SB (2017). Pathogenic variability of

262   *Meloiodogyne incognita* populations occurring in pepper-production greenhouses in Israel toward

263   *Me1*, *Me3* and *N* pepper resistance genes. Plant Disease 101(8): 1391-1401.

264   https://doi.org/10.1094/PDIS-11-16-1667-RE

265

266   Coyne DL, Cortada L, Dalzell JJ, Claudius-Cole AO, Haukeland S, Luambano N, Talwana H. Plant-

267   parasitic nematodes and food security in sub-Saharan Africa. Annual Review of Phytopathology 56:

268   381-403. https://doi.org/10.1146/annurev-phyto-080417-045833

269

270   Davies LJ, Elling A (2015). Resistance genes against plant-parasitic nematodes: a durable control

271   strategy? Nematology 17(3): 249-263. https://doi.org/10.1163/15685411-00002877

272

273   Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR

274   (2013). STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29(1): 15-21.

275   https://doi.org/10.1093/bioinformatics/bts635

276

277   Enright AJ, John B, Gaul U, Tuschl T, Sander C, Marks DS (2003)MicroRNA targets in *Drosophila*.

278   Genome Biology 5(1): R1. https://doi.org/10.1186/gb-2003-5-1-r1

279

280   Friedländer MR, Mackowiak SD, Li N, Chen W, Rajewsky N (2012). MiRDeep2 accurately identifies

281   known and hundreds of novel microRNA genes in seven animal clades. Nucleic Acids Research 40(1):

282   37–52. https://doi.org/10.1093/nar/gkr688

283

284   Howe KL, Bolt BJ, Cain S, Chan J, Chen WJ, Davis P, et al. (2015). WormBase 2016: expanding to

285   enable helminth genomic research. Nucleic acids research 1217.

286   https://doi.org/10.1093/nar/gkv1217

287

288   Iwakawa H-O, Tomari Y (2015). The functions of microRNAs: mRNA decay and translational

289   repression. Trends in Cell Biology 25(11): 651-665. https://doi.org/10.1016/j.tcb.2015.07.011

290

291   Kawecki TJ, Lenski RE, Ebert D, Hollis B, Olivieri I, Whitlock MC (2012). Experimental evolution.

292   Trends in Ecology and Evolution 27(10): 547-560. https://doi.org/10.1016/j.tree.2012.06.001

293

294   Lee JS, Shih PY, Schaedel ON, Quintero-Cadena P, Rogers AK, Sternberg PW (2017). FMRFamide-like

295   peptides expand the behavioral repertoire of a densely connected nervous system. Proceedings of

296   the National Academy of Sciences U S A. 114(50): E10726-E10735.

297   https://doi.org/10.1073/pnas.1710374114

298

299   Li B, Dewey CN (2011). RSEM: accurate transcript quantification from RNA-Seq data with or without

300   a reference genome. BMC bioinformatics 12(1): 323.

301   https://doi.org/10.1186/1471-2105-12-323

302

303   Love MI, Huber W, Anders S (2014). Moderated estimation of fold change and dispersion for RNA-

304   Seq data with DESeq2. Genome Biology 15(12):550. https://doi.org/10.1186/s13059-014-0550-8

305

306   Mangone M, Manoharan AP, Thierry-Mieg D, Thierry-Mieg J, Han T, Mackowiak SD, Mis E, Zegar C,

307   Gutwein MR, Khivansara B, Attie O, Chen K, Salehi-Ashtiani K, Vidal M, Harkins TT, Bouffard P, Suzuki

308   Y, Sugano S, Kohara Y, Rajewsky N, Piano F, Gunsalus KC, Kim JK (2010). The landscape of *C. elegans*

309   3'UTRs. Science 329(5990): 432-5. https://doi.org/10.1126/science.1191244

310

311   Morris R, Wilson L, Sturrock M, Warnock ND, Carrizo D, Cox D, Maule AG, Dalzell JJ (2017). A

312   neuropeptide modulates sensory perception in the entomopathogenic nematode *Steinernema*

313   *carpocapsae*. PLoS Pathogens 13(3): e1006185. https://doi.org/10.1371/journal.ppat.1006185

314

315   Rathan M, Gong J, Li Z, Liu J, Xu XZS. MicroRNA regulation of nAChR expression and nicotine-

316   dependent behaviour in *C. elegans*. Cell Reports 21(6): 1434-1441.

317   https://doi.org/10.1016/j.celrep.2017.10.043

318

319   Szitenberg A, Salazar-Jaramillo L, Blok VC, Laetsch DR, Joseph S, Williamson VM, Blaxter ML, Lunt DH

320   (2017). Comparative genomics of apomictic root-knot nematodes: hybridization, ploidy and dynamic

321   genome change. Genome Biology Evolution 9(10): 2844-2861. https://doi.org/10.1093/gbe/evx201

322

323   Trudgill DL, Blok VC (2001). Apomictic, polyphagous root-knot nematodes: exceptionally successful

324   and damaging biotrophic root pathogens. Annual Review of Phytopathology 39: 53–77.

325   https://doi.org/10.1146/annurev.phyto.39.1.53

326

327   Wang Y, Mao Z, Yan J, Cheng X, Liu F, Xiao L, Dai L, Luo F, Xie B (2015). Identification of microRNAs in

328   *Meloidogyne incognita* using deep sequencing. PLoS One 10(8): e0133491.

329   https://doi.org/10.1371/journal.pone.0133491

330

331   Warnock ND, Wilson L, Canet-Perez JV, Fleming T, Fleming CC, Maule AG, Dalzell JJ (2016).

332   Exogenous RNA interference exposes contrasting roles for sugar exudation in host-finding by plant

333   pathogens. International Journal of Parasitology 46(8):473-477.

334   https://doi.org/10.1016/j.ijpara.2016.02.005

335

336   Warnock ND, Cox D, McCoy C, Morris R, Dalzell JJ (2018) Transcriptional variation and divergence of

337   host-finding behaviour in *Steinernema carpocapsae* infective juveniles. BioRχiv

338   https://doi.org/10.1101/272641

339

340   Xuan Z, Manning L, Nelson J, Richmond JE, Colon-Ramos DA, Shen K, Kurshan PT (2017). Calrinet

341   (CLA-1), a novel active zone protein required for synaptic vesicle clustering and release. eLife

342   6:e29276. https://doi.org/10.7554/eLife.29276

343

344   Zhang L, Hammell M, Kudlow BA, Ambros V, Han M (2009). Systematic analysis of dynamic miRNA-

345   target interactions during *C. elegans* development. Development 136(18): 3043-3055.

346   https://doi.org/10.1242/dev.039008

347

348   Zhang Y, Wang Y, Xie F, Li C, Zhang B, Nichols RL, Pan X (2015). Identification and characterization of

349   microRNAs in the plant parasitic root-knot nematode *Meloidogyne incognita* using deep sequencing.

350   Functional & Integrative Genomics 16(2): 127-42. https://doi.org/10.1007/s10142-015-0472-x